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Detection of *Anaplasmataceae* in ticks collected in Morocco

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Bacteria within the *Anaplasmataceae* include Gram-negative intracellular bacteria that have been known for a long time as the agents of veterinary diseases. However, in the recent years, five members of *Anaplasmataceae* have been reported to infect humans, including *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, *Ehrlichia chaffeensis*, the agent of human monocytic ehrlichiosis, and *E. ewingii*, *E. canis* and *Neorickettsia sennetsu* [1].

The abundance of ticks in North Africa represents potential risks for animal and human public health. However, agents within the *Anaplasmataceae* family have been poorly investigated in North Africa, including in Morocco. In this work, we analysed ticks collected in this country for evidence of infection by *Anaplasmataceae*.

MATERIALS AND METHODS

A total of 167 ticks were collected by blanket dragging of the vegetation and by direct removal from dog and cattle in different regions of Morocco, including Kenitra, Marrakech, Taza, Casablanca and Meknès. All the ticks were identified by one of us (MS) and stored in 70% ethanol until they were further processed. DNA was extracted and *Anaplasmataceae* were detected by *rrS* PCR using primers Ehr16SD and Ehr16SR, which amplified a 345 bp of DNA fragment [2] We used sterile water and DNA of uninfected tick as negative controls and DNA of *A. phagocytophilum* was used as positive control of *rrS* PCR of *Anaplasmataceae*. In order to identify the detected *Anaplasmataceae*, PCR products were amplified and purified, and then the DNA sequenced as previously described [2]. All obtained sequences were assembled by Chromas Prov.1.34 (Chromas Prov 1.34 Technelysium Pty Ltd., Atlanta, GA, USA) then compared in GenBank as previously described. The phylogenetic tree is produced by software MEGA 4 (The Biodesign Institute, Tempa, FL, USA) after aligning the sequences found with the sequences in GenBank by CLUSTAL W 1.83 (Welcome Trust, Cambridge, UK).

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RESULTS

Tick species were identified immediately after collection and revealed 125 of *Rhipicephalus sanguineus*, 25 of *Rh. bursa*, three of *Rh. turanicus* and 14 of *Ixodes ricinus*. After the sequencing, several *Anaplasmataceae* were identified in 19 ticks tested positive by *rrS* PCR for *Anaplasmataceae* allowing sequence analysis. The sequence identified in one *I. ricinus* specimen collected from vegetation in the Taza region was found to be closely related to the corresponding sequence of '*Candidatus* Midichloria mitochondrii' (GenBank accession number AJ566640), with 99.1% (342/345) base pair similarity. A total of 16 ticks were found to harbour *Wolbachia* spp. The amplified sequences from these ticks showed 97–99% similarity with the corresponding sequence of *W. pipientis* (GenBank accession number DQ981321). These ticks include 12 *Rh. sanguineus* (five collected from dogs in Marrakech, five from Kenitra and two from Meknes region), three *Rh. bursa* (two from Kenitra and one from Meknes) and one *Rh. turanicus* collected in the Kenitra region. One *Rh. sanguineus* collected from dogs in the Casablanca region was found to be unambiguously infected with *A. platys*, (100% similarity with the corresponding sequence of *A. platys* GenBank accession number AF156784). Finally, the sequence identified in one *Rh. bursa* collected in Kenitra was 100% similar to those of *A. centrale*, *A. ovis* and *A. marginale* (GenBank accession number, EF520690, EF587237, DQ341370, respectively).

DISCUSSION

For the first time in Morocco, we have identified by molecular tools several bacteria of the *Anaplasmataceae* family in ticks. *A. platys* is a parasite

of canine platelets and dogs naturally infected with this agent do not necessarily show clinical signs. *A. platys* has been reported throughout the world, including in Europe, Asia, Australia, America and Africa. In 2003, it had been detected for the first time in *Rh. sanguineus* from Africa (Democratic Republic of Congo) by Sanogo *et al.* [2]. The second bacterium detected here was similar to *A. centrale*, *A. marginale* and *A. ovis*. The size of the fragment that was sequenced was not big enough to differentiate among these three species. All these three species are known as targeting the red blood cells of ruminants, *A. marginale* being the most pathogenic, *A. centrale* being less pathogenic in cattle and *A. ovis* being moderately pathogenic in small ruminants. Although anaplasmosis is more frequently associated with haemolytic anaemia in goats, *A. ovis* can also cause disease in sheep. In the Old World, *A. ovis* has been associated with *R. bursa* [3]. However, Fuente *et al.* have identified *A. marginale* in the salivary gland of *R. bursa* removed from Iberian red deer [4]. '*Candidatus* Midichloria mitochondrii' is an intramitochondrial bacterium that has been detected in Europe in *I. ricinus* ticks. Its pathogenicity for humans or animals is unknown [5]. Finally, *Wolbachia* spp. are a group of bacteria associated with arthropods (insects, ticks, spiders, etc.) as well as filarial nematodes. Basically, a single species has been properly described – *W. pipientis* [1]. More work is needed for a precise identification of the *Anaplasmataceae* identified in this work. These preliminary results, however, increase our knowledge about the prevalence of *Anaplasmataceae* pathogens in Morocco and may help to implement measures to control transmission to humans and animals in this region.

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